(12) UK Patent Application (19) GB (11) 2 187 035 (13) A

(43) Application published 26 Aug 1987

(21) Application No 8601856

(22) Date of filing 27 Jan 1986

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(51) INT CL[4]
G01N 27/62

(52) Domestic classification (Edition I)
H1D 12B47Y 12B4 19X 19Y 21B 21C 21X 51 9L 9Y M
G1N 19B2X 19F1B 19X1 24A 24B3EX
U1S 1289 1296 1303 G1N H1D

(56) Documents cited
None

(58) Field of search
H1D
G1N
Selected US specifications from IPC sub-class G01N

(54) Pyrolysis mass spectrometer disease diagnosis aid

(57) Micro-organisms may be rapidly identified by comparing the mass distribution of molecular fragments of the micro-organisms with those of known organisms. In one embodiment, a pyrolysis process produces the fragments which undergo an ionisation process to enable a mass distribution of the fragments to be distinguished by a quadrupole mass spectrometer and recorded to form a mass spectrogram of primary fragments. An integral computer is used to unravel the composition of the sample spectrogram by taking reference to a data bank of species spectrograms and using an algorithm to elicit the identities of the constituent micro-organism species.

In a preferred embodiment, secondary fragmentation generated by collision activated dissociation augments the first fragmentation stage and serves to reveal the structure of the primary fragments, thus enabling complex medical samples to be analysed for their constituent micro-organisms. The invention is capable of identifying micro-organisms embedded in tissue material together with antibiotic, antigenic, toxin and other biological materials.

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The drawing(s) originally filed was (were) informal and the print here reproduced is taken from a later filed formal copy.

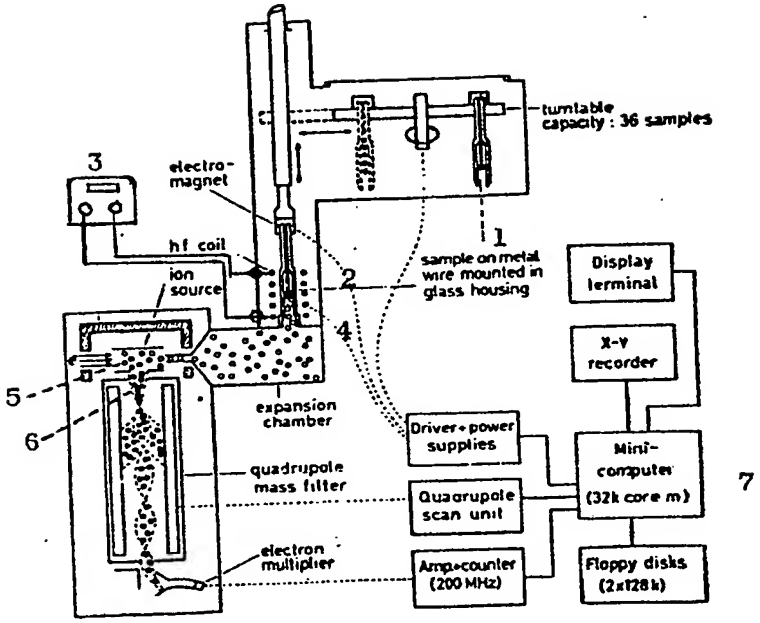

FIG. 1. — *Schematic representation of the fully-automated Curie-point pyrolysis mass spectrometry system*

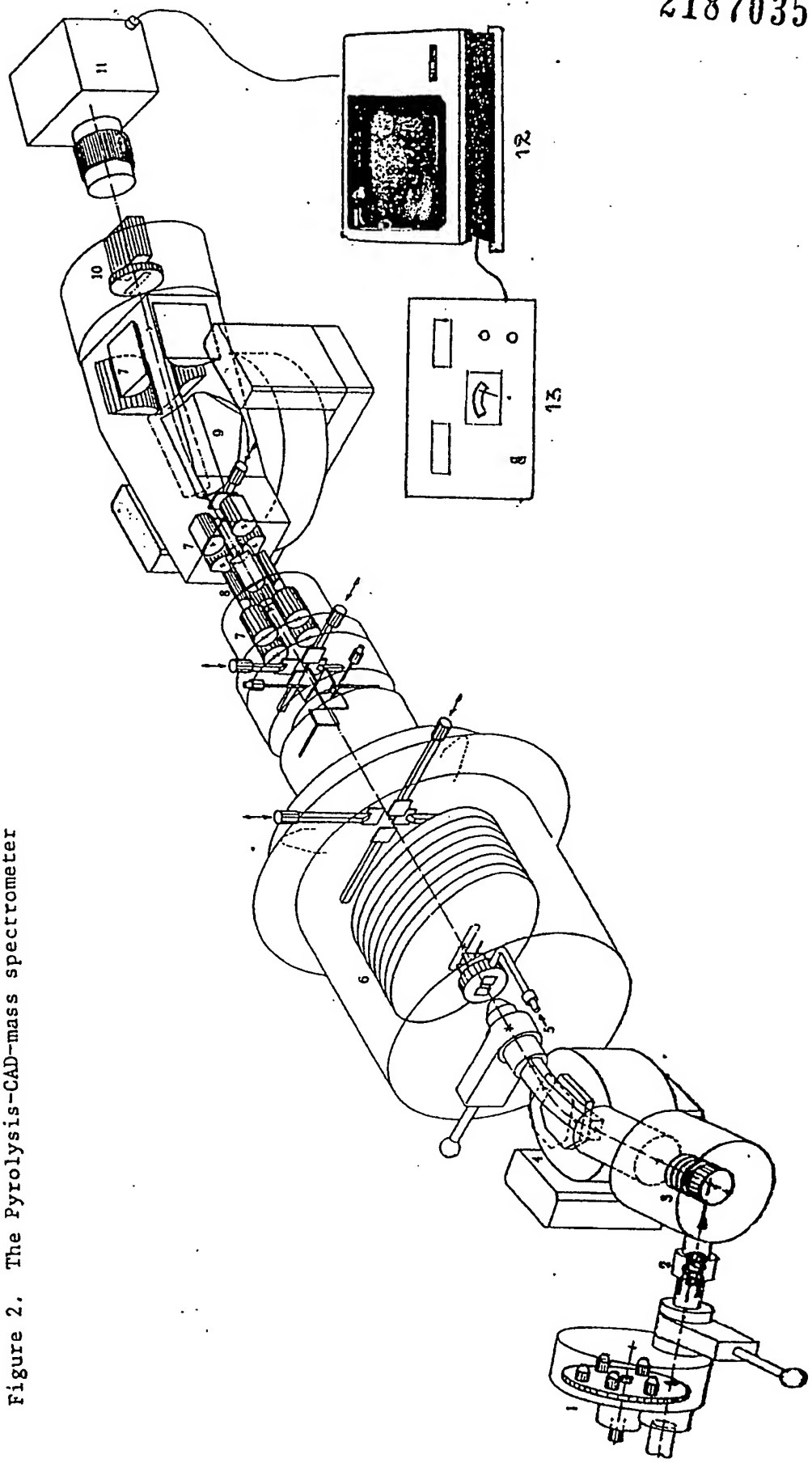

Figure 2. The Pyrolysis-CAD-mass spectrometer

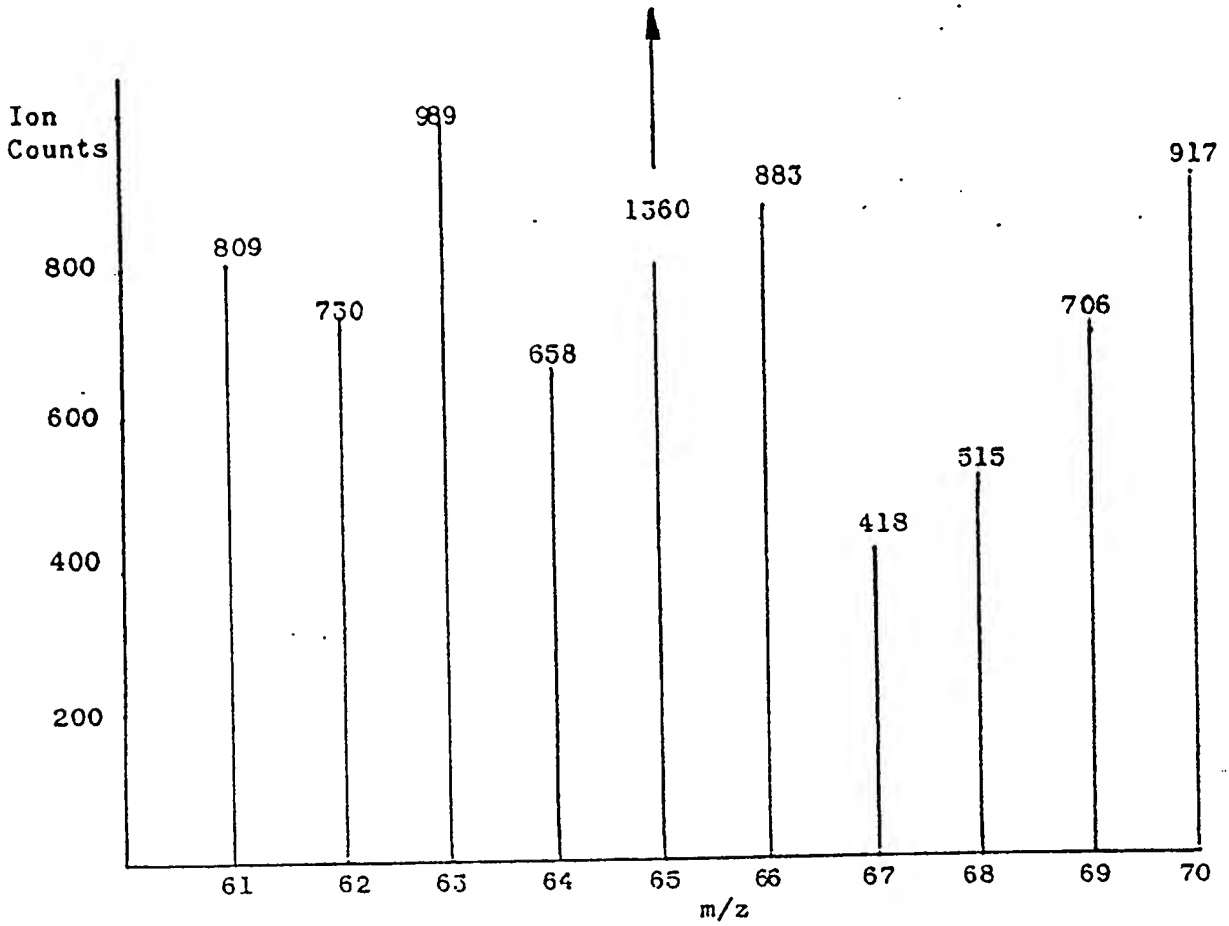

Figure 3 Illustrative primary fragmentation data which represents the spectrogram of a sample suspended in a low molecular weight liquid.

Figure 4

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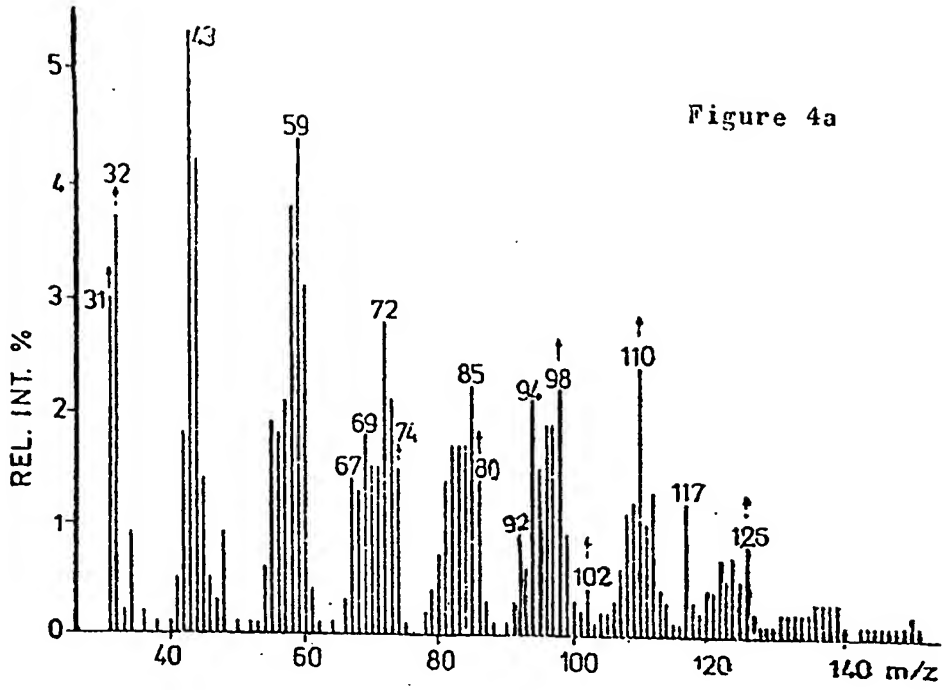

Figure 4a

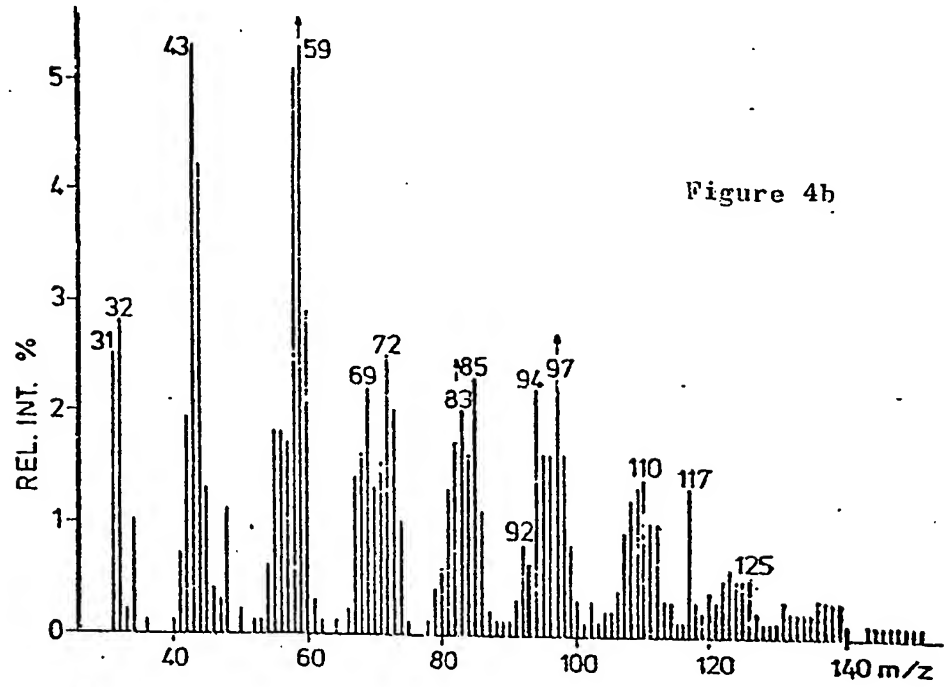

Figure 4b

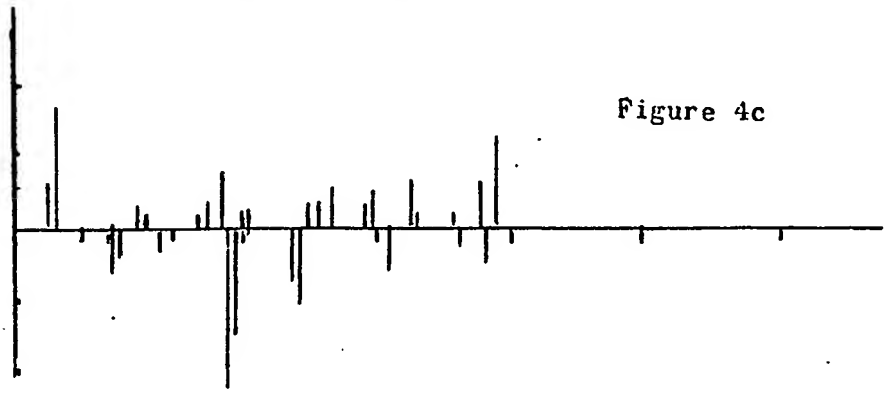

Figure 4c

SPECIFICATION

Pyrolysis mass spectrometer disease diagnosis aid

This invention relates to an improvement in detecting the presence of micro-organisms which act as the causative agents of disease in humans where the said micro-organisms are to be found in human body fluids, human tissue material and other materials with which human activity is associated, such as water, liquids, foodstuffs and other materials that are taken into the human body for the purpose of maintaining, enhancing or prolonging the well being of the human.

Present methods of analysis of micro-organisms are unsatisfactory due to the slow response time of the analysis techniques which are applied after suitable samples have been drawn from the sources of interest.

The analysis techniques that are currently used to identify the causative agents of disease reply heavily upon the medical microbiological culture methods and techniques that have been developed over the years to identify relevant micro-organisms.

These culture methods are time consuming, costly and tend to provide data which has reduced medical value on account of the time lapses that culture techniques require before results become available for medical assessment and interpretation. These time lapses range from a few hours to days and sometimes weeks, during which time the medical condition of a patient can alter significantly, thus placing the patient at risk by virtue of obscuring the immediate medical condition from a medical clinician or surgeon and delaying diagnosis.

The aim of this invention is to reduce the time required for sample analysis to about 2 minutes, and produce listings of microbial species contained within samples together with numerical estimates of species occurence for the purposes of assessing the medical conditions of patients, assisting the diagnosis processes, and aiding the selection of medical treatment.

The invention comprises a pyrolysis mass spectrometer, a collecting medium and a computer which are used in combination together to accomplish analyses of the microbial contents of samples relevant to the control of disease. This task is accomplished by thermally degrading relevant sample material in a highly controlled manner and recognising the resulting pyrolysis fragmentation patterns.

In essence, the pyrolysis mass spectrometer instrument generates a record of the fragmentation pattern called a mass spectrogram, and the computer analyses these data and unravels the composition of the spectrogram to reveal which species are present in the sample. This process is termed spectral deconvolution.

The pyrolysis mass spectrometer is a very sensitive instrument for determining the presence of micro-organisms and other macromolecules, and is capable of responding to small amounts of sample material. Typically, five micrograms of material are sufficient for an acceptable analysis to be carried out.

In order to better understand the working of the invention an explanation of the pyrolysis mass spectrometer is given together with an indication of the nature of the pyrolysis process.

The pyrolysis process is carried out first by means of a collecting medium which is made of a Curie point metal alloy that is heated by electromagnetic induction to a temperature that is regulated by the alloy composition, and which therefore controls the temperature at which the pyrolysis process takes place and also determines the reproducibility with which it takes place. The electromagnetic induction is exited by a suitable device e.g. an RF generator.

To persons skilled in the art of pyrolysis mass spectrometry, a preferred temperature for pyrolysing micro-organisms is known to be about 510° that is established in less than one second with a temperature reproducibility of 0.1°C in an enclosure in which a vacuum of the order of 1×10^{-6} torr has been maintained immediately prior to the pyrolysis process being carried out.

The pyrolysis fragments are conducted to an ionisation chamber where low energy electrons are allowed to impinge upon the said fragments and cause them to become singly charged particles, i.e. each fragment acquires one electron from the impingements in which electrons having an energy of about 14 eV (electron volts) are used for the purpose.

After ionisation, an electric drift field may be used to ensure that the ionised particles or fragments, move to the mouth of the mass spectrometer where the fragments are distinguished one from another in terms of mass-charge ratio. Effectively, this means that all ionised fragments having the same mass are separated at one instant in time from all those having a different mass, and are then counted together by the spectrometer to form a mass-ion count (i.e. intensity) record of the said fragments.

By repeating this operation for all the other masses that the spectrometer is capable of distinguishing, a complete record may be obtained of the intensity of each mass. A complete record of this kind is called a mass spectrogram.

Each different species of macromolecule and micro-organism has a molecular structure that is unique to that species, and this structure is unambiguously represented in the mass spectrogram with sufficient clarity and distinction as to make species identification possible and reliable. Spectrograms of this quality may be obtained under the aforementioned pyrolysis conditions provided that the walls of the chamber and conductance tubulations are maintained at a temperature of 150°C as is the case for this invention.

These conditions ensure that the quintessential features of the fragments and fragmentation patterns are

preserved for a time interval of at least 10 (ten) seconds during which the ionisation process and mass spectrometer measurements may be completed. These conditions reduce the probability of physico-chemical interactions between fragments, and also between f ragments and their environment, to acceptable levels so as to inhibit the production of misleading mass spectrograms. Such interactions sometimes occur in small numbers, but their occurrences are small enough to be accommodated in the data analysis and enable safe and reliable species identifications to be made by means of the invention.

An illustrative diagram of a tandem mass spectrometer is shown in Figure 1. The sample material 1 is shown mounted upon the needle shaped support 2 which is made of a Curie-point metal alloy that responds to RF (radio frequency) electromagnetic induction to heat the sample material to 510°C. The RF generator 3 is shown to be connected to the pyrolysis chamber by means of special vacuum seals which connect electricity into the vacuum enclosure in which the pyrolysis process and other processes and measurements take place.

The walls of the pyrolysis chamber 4, the connecting tubulations and all other chambers and components are all maintained at a temerature of 150°C, and in a preferred form of the invention all the said walls are coated on the vacuum side with a thin layer of noble metal such as gold (Au), except those areas where electrical shorting would occur. These conditions prevent the unwanted settlement of fragments upon the walls supports and other parts of the apparatus prior to the passage of the said fragments into the mass spectrometer regions.

Electrons are emitted from a source such as a filament made of thoriated tungsten wire, rhenium, platinum or other electron emitting material which is well known in the art of manufacturing electron discharge devices. These electrons are accelerated in a low electric field which imparts energy to the said electrons of about 14.0 electron volts. These electrons impact upon the pyrolysis fragments and produce singly ionised fragments without causing further fragmentation. This low energy electron impact ionisation takes place in the confines of the ionisation chamber 5, and another electric field has the effect of conducting the ionised fragments into the entrance of the mass spectrometer 6, where the number of fragments having a given mass may be detected and recorded.

The mass spectrometer is an instrument that is well known in the scientific disciplines of physics and chemistry and does not need to be described in this document in any detail. It is sufficient to remark that mass spectrometers take a number of different forms by employing different combinations of electrostaic and magnetic focussing elements in the electron-optical design of the spectrometer. Different advantages and disadvantages pertain to different designs of spectrometer, though the quadrupole mass spectrometer is used in this invention as a preferred form of spectrometer on account of the relatively low cost and acceptable mass resolution which is obtained at repetition rates that are suitable for the purpose of generating mass spectrograms of the required quality.

In a preferred form of the invention the spectrometer 6 scans all the masses that the instrument distinguishes and premanently records the values by means of the computer 7 which generates an electonic image onto a computer tape or disc from which a printed output may be obtained. Thus the mass spectrogram of a sample may be accomplished by means of the invention in one of the forms that the invention may take.

The resulting spectrogram is composed of primary fragmentation data which is used in one form of the invention for the identification of microbial spcies in medical samples that have undergone a preferred preparation process prior to analysis. These data may be used for the identification of microbial species in rather limited circumstances such as simple mixtures of species suspended in low molecular weight fluids, so that the practical utility is restricted to the comparative studies of species, epidemiology and the like.

Secondary fragmentation data are gathered and used in a preferred form of the invention for the purposes of identifying microbial species in compex medical samples. The generation of these data is accomplished by allowing the ions formed from the primary fragmentation process to pass into another chamber containing a partial pressure of a chemically inert gas so that collision processes occur at a significant rate between the ions and inert gas molecules which cause secondary fragmentation of the said ions to occur. This latter process is termed 'collision activated dissociation' (C.A.D.). These secondary fragments are then distinguished for their mass by a second mass spectrometer 9* which reveals further information about the molecular structure of the micro-organism and the computer 12* (*See Figure 2 and the description of the Pyrolysis-CAD-mass spectrometer) then records the fragment counts to form a secondary fragmentation mass spectrogram. Secondary fragmentation data are used in this invention to unravel the composition of medical samples containing complex mixtures of micro-organisms, body fluids, antibiotic, antigenic, toxin and other materials including those that may be used in acquiring the said samples. Safe identifications of microbial species are very difficult to achieve using primary fragmentation data alone so that the information that CAD provides is essential for the working of this invention where complex samples are concerned. These samples include those drawn from human patients, sources of medical materials and other sources of natural samples which have a bearing on the medical well being of human communities.

Primary fragmentation data may be used for preliminary assessments of epidemiology, the comparative studies of species together with the detection of the presence of micro-organisms in gases, liquids and on the surfaces of solids. In favourable circumstances the onset of species mutations can be detected by using primary fragmentation investigations.

In the preferred form of the invention the collision activated dissociation process is achieved by passing the ions that have been sensed by the first mass spectrometer into the CAD chamber where the secondary

fragmentation takes place. After fragmentation the post acceleration field 6 conducts the secondary fragments to the second mass spectrometer 9 where the said fragments are then detected by a simultaneous detector device 10 and the resulting data are recorded by the computer 12, which then has a record of the primary data and the manner in which the primary ions fragment. This latter information is gathered by using time corelation between the data that the two spectrometers generate, i.e. the time of flight that lapses after a given ion has left the environs of the first spectrometer until it is detected by the second spectrometer has to be taken into account in order to link the fragmentation data correctly. This correlation is performed by the computer control program.

The invention may be applied to two different classes of medical samples. In the first of these little or no chemical or biological interaction occurs between the micro-organisms and the medium in which they are suspended, as for instance in the case of body fluid samples such as blood, urine and lymph. For this class, the pyrolysis mass spectrogram (primary fragmentation) of the sample basically comprises the spectrograms of the suspension medium and the constituent micro-organisms in a simple combination reflecting the relative proportions of the said constituents. Identifying the constituent species is accomplished by the computer program which is used by the invention to analyse the spectrogram data to determine which species are represented in the sample spectrogram, as well as the species occurences.

Separating the microbial content of this class of sample from the suspension medium and replacing it with a low molecular weight fluid has the advantage of reducing the data analysis effort that is required to unravel the composition of the sample spectrogram. this advantage arises because the spectrogram data of the natural suspension medium is eliminated from consideration and the spectrogram data of the new medium can be safely ignored. A further advantage of using this practice is that primary fragmentation instruments may be safely used for species identification at low costs for samples containing up to ten different species.

The preferred form of the invention may be used for analysing samples in which micro-organisms are embedded in tissue material so that microbial separation is impractical, and in addition other constituents may be present such as body fluid, antibiotic, antigenic, toxin and other materials which have to be distinguished in the mass spectrogram of the sample. The invention minimises the data analysis effort by utilising the patient's case notes in those situations where this is possible. These data specify which type of tissue has been selected for analysis and other data include which antibiotics have been administered and by implication which endotoxins may be present, all of which serves to reduce the data analysis task.

The modus operandi for analysing spectrograms of the first class of medical sample is to apply a preprocessing computer program which searches a data bank of spectrograms and select relevant spectrograms that are compatible with sample spectrogram. After this stage a heuristic program elicits a set of test values to represent trial values for species occurence, these values are passed over to a third program which refines these values in order to synthesise numerically the corresponding values that appear in the sample spectrogram. Synthesised solutions are refined until the differences between the spectrogram values and the corresponding values of the solution are less than 1% which represents the error magnitudes of the instrumentation. This order of difference magnitude is achieved by operating these two programs on an interplay basis passing the development of a solution back and forth until the error threshold level is achieved. A fourth program tests a solution that has reached this level for similarity with respect to the sample spectrogram and chooses the solution which most closely resembles the sample spectrogram, where the development of more than one solution occurs. The third program takes the form of the Gauss-Seidel technique for solving systems of linear simultaneous equations, but the standard form has been modified to prevent zero coefficients appearing and solution convergence is augmented by an over-relaxation technique.

The spectrograms of complex medical samples are unravelled by a fifth program which compares the spectrograms of the relevant tissue material that has been infected with viral or bacterial micro-organisms: Provision is made to detect the spectrogram data pertaining to antibiotic, antigenic and other biological materials in such samples. An illustrative example of the principles of the method is shown in Figure 4 this figure shows how the spectrogram of a given tissue material Figure 4a is altered as a result of virus infection Figure 4b and the difference spectrogram that results from subtracting the spectrograms of Figure 4a and Figure 4b. The difference spectrogram Figure 4c is then subjected to computer aided analysis which is similar to that mentioned above.

In yet another aspect of the invention the presence of mutant forms of microbial species isolates may be detected in samples of species isolates by revealing the small changes in molecular structure that mutant forms and strains exhibit. The mutant structures will cause in some cases, different chemical and biological inter-actions to take place with respect to given body fluids, antibiotic and other materials with which the said mutants come into contact.

In another aspect of the invention there is provided a means for connecting an instrument-computer combination to another of the same kind or similar entity. Interconnecting a number of these instrument-computer combinations enables a localised network to be created which may be extended to form an area, regional and eventually a national network. Such a network has the capacity to assist in the control of disease in both technical and administrative aspects of the said control, since it may be used to co-ordinate the application of disease counter-measures in areas, regions, nationally and even internationally through the aegis of the national networks where they exist. It is a feature of the invention that immediate access to disease information is potentially available under computer command over a wide area

of disease control for the purposes of formulating said counter-measures and predicting the spread of disease. In principle, the dissemination of this information may begin even as the presence of micro-organisms is confirmed in relevant medical samples so that an immediate response may be made to changing situations of disease control.

The invention enables the efficiency of couter-measures to be assessed and the prediction of disease movement to be updated, as the events of interest take place.

Figure 3 is an illustrative primary fragmentation spectrogram representing a simple mixture of four microbial species that was analysed in a low molecular weight suspension medium, and which therefore reduces the data content and analysis effort to a minimum for the convenience of the reader*. Data relating to masses below 60 m/z are ignored since these data represent the suspension medium alone.

Table comprises the spectrogram data of the species that have been selected for consideration as component species of the mixture. The bottom row of values are the mass-ion or 'channel' counts that the instrument-computer combination records for the analyst to unravel, aided by the computer algorithm in order to find the values for species occurence and relative abundance which are shown in the last two columns of the table.

In this illustrative case the values for the ion count data are not entirely exact so that a residual error of about 1 % is apparent. This order of error magnitude is representative of pyrolysis mass spectrometry as is currently practiced.

* In reality the range of masses that currently feature in instruments extends from 100 to about 400 for Py-CAD-MS spectrometers.

TABLE 1

m/z	*61*	*62*	*63*	*64*	*65*	*66*	*67*	*68*	*69*	*70*	*No*	α
Ax	45	27	32	21	12	9	6	17	11	4	12	.3077
Lc	15	14	31	29	34	27	36	32	20	17	7	.1795
Pg	7	12	16	10	31	32	4	6	24	39	17	.4359
zi	16	8	41	13	15	12	9	16	7	31	3	.0769
Ions	808	735	989	658	1370	883	418	515	706	917	39	1.0000

The letters Ax, Lc, Pg and zi denote the species identification mark.

The ion counts from the spectrogram are found by multiplying the column coefficients by the corresponding row number located in the column marked No, e.g. for the first column we form the sum of:

$45 \times 12 + 15 \times 7 + 7 \times 17 + 16 \times 3 = 812$ which is close to 809 and the difference is due to instrument error.

The preferred form of the invention joins pyrolysis fragmentation with collision activated dissociation (Py-CAD-MS) mass spectrometry in which Curie-point pyrolysis provides the primary fragmentation products. A schematic diagram of the Curie-point pyrolysis tandem mass spectrometer is shown in Figure 2. The design and operating conditions for the pyrolysis inlet system are similar to those previously described for the Py-MS system of Figure 1. This preferred spectrometer consists of an automated sample changer 1, a pyrolysis temperature of 510°C in a temperature rise time of 0.1 seconds and a heating time of 1.0 second. An expansion chamber connects the pyrolyser 2 with the ionisation chamber 3 in which the wall temperatures are maintained at 150°C and ions are produced by low energy electrons (14.0 eV) electron impact ionisation, these ions are accelerated to 6 kV and are mass selected by the static sector magnet 4 where a 50° deflection angle is achieved together with a main radius of 160 mm and a mass resolution of about 1,000. In this description mass spectrometer and mass filter are used synonymously for the mass selection devices.

The selected ion-beam is guided into the collision activated dissociation (CAD) cell 5 of length 10 mm in which helium is retained at a pressure of about 0.1 Torr where ionised fragment dissociation takes place. Post acceleration sector 6 applies up to 20 kilo-volts to the fragments in order to reduce the effects of the kinetic energy spread in fragment energy distributions which accompany the said dissociation on account of the release of translational energy attendant upon this type of dissociation process. This post acceleration has the effect of improving mass resolution so that the beam of fragments may be analysed by an electric quadrupole lens 7 working with a magnetic quadrupole lens 8 and a second analysing magnet 9 in order to effect a suitable mass dispersion of the secondary fragments. The second analysing magnet provides a deflection of 15° in angle with a main radius of 750 mm and a mass resolution of 600. A simultaneous ion detection arrangement 10 is employed which consists of a double channeltron electron multiplier array (CEMA) having a diameter of 75 mm and 25 um diameter channels which offers an electron gain of about 2,000. The leectric and magnetic quadrupole lenses and the analysing magnet provide adequate ion beam focussing and suitably adjustable mass dispersion which typically ranges in mass ratio (highest to lowest) from 12 to 1, down to 1.3 to 1 and where a mean value of 4 to 1 is used for most purposes. The CEMA is electron-optically connected to a phosphor screen which responds to ion bombardment to provide optical radiation as an output which represents the intensity of the ion beam mass distribution as distinguished by

the analysing magnet 9. This radiation stream is optically coupled to a fibreoptic block which is incorporated into the simultaneous detector 10. A camera objective lens images the spectrum which appears at the output side of the fibre-optic onto a photodiode array 11 having a 25 mm width, a height of 0.43 mm and 1024 channels, which is used in an integrating mode. This enables the spectrum to be summed for 4 seconds (typically) before being recorded on the computer memory or disc 12. Each sample is analysed and recorded for two successive integration periods to ensure that gross errors are detected by a comparison between the records. This practice requires a measurement time of 8 seconds to complete the data acquisition for each sample, and sample analysis periods of less than two minutes may be achieved when the time required for the fragments of one sample to clear from the spectrometer are reduced to less than 100 seconds.

Figure 2 Annotations:

(1) Sample changing device [turntable]
(2) Curie point pyrolyser
(3) Ion source
(4) Selection magnet
(5) Collision cell
(6) Post acceleration section
(7) Electric quadrupole lenses
(8) Magnetic quadrupole lens
(9) Analysing magnet
(10) Simultaneous detection system
(11) Vidicon camera
(12) Computer
(13) Electronic Controller unit

CLAIMS

1. An instrument comprising a sample holder in the form of a needle or foil made from a Curie-point metal alloy by which a micro-organism may be pyrolysed to produce molecular fragments in a partial vacuum and which are then ionised by means of an associated ionisation chamber after which the masses of thesaid fragments are measured by a mass spectrometer to provide primary fragmentation data which is used to identify the said micro-organism by virtue of a prior knowledge of the pattern of fragments that is characteristic of the molecular structure of the said micro-organism.

2. An instrument as claimed in Claim 1 which is fitted with a computer that is used to store or retain the fragmentation patterns of a number of different micro-organisms for the purposes of identifying the component micro-organisms of mixtures of microbial species as would appear in human body fluid samples drawn from infected patients.

3. An instrument and computer combination as claimed in Claim 2 into which a secondary fragmentation facility is incorporated together with a second mass spectrometer to provide secondary molecular fragmentation data for the purposes of identifying the causative agents of disease in complex medical samples drawn from human patients in whose tissue materials the said agents are expected to be found, possibly in company with antibiotic and other macromolecular materials and substances.

4. An instrument and computer combination as claimed in Claim 3 which may be used during the progress of surgery or medical investigations to identify the nature of tissue abnormalities, and also to identifying the malignant or benign nature of tumours or other deviant materials in the human body.

5. An instrument and computer combination as claimed in Claim 3 which rapidly detects and identifies within a time interval of about two minutes, micro-organisms in samples of human body fluids, human tissue and materials and substances which come into contact with the human body during the practice of medicine.

6. An instrument and computer combination as claimed in Claim 3 may be interconnected through the said computer to a multiplicity of similar or identical entities to form an area, regional or national network of micro-organism identification stations which may be used to exercise the control of disease in the catchment areas that the said networks are required to serve.

7. The invention as claimed in Claim 6 may be interconnected to the national network of another country to form an international network to exercise control over the spread of diseases such as rabies, AIDS, tuberculosis and the like, from one land tract to another.

Amendments to the claims have been filed, and have the following effect:-
(a) Claim 2 above has been textually amended.

2. An instrument as claimed in Claim 1 which is fitted with or connected to a computer that is used to store or retain the fragmentation patterns of a number of different micro-organisms for the purposes of identifying the component micro-organisms of mixtures of microbial species as would appear in human body fluid samples drawn from infected patients.

Printed for Her Majesty's Stationery Office by Croydon Printing Company (UK) Ltd, 7/87, D8991685.
Published by The Patent Office, 25 Southampton Buildings, London WC2A 1AY, from which copies may be obtained.